AMENDMENTS TO THE SPECIFICATION

A substitute specification in English is attached hereto including the amendments listed below:

Please insert the following paragraph beginning on page 1, beneath the title:

CROSS-REFERENCES

This application is a 371 National Phase application of International Patent Application Serial No. PCT/JP2004/018413, filed December 9, 2004, which claims priority to Japanese Patent Application No. 2003-415779, filed December 12, 2003, which are incorporated herein by reference in their entirety noting that the current application controls to the extent there is any contradiction with any earlier applications and to which applications we claim priority under 35 USC §120 and 119.

Please replace the paragraph beginning on page 3, line 25 with the following rewritten paragraph:

Thus, it was proven for the first time that RAGE overexpression could enhance the onset and progression of diabetic nephropathy at the individual level. RAGE was identified as a nephropathy-susceptible gene and it was confirmed that RAGE/iNOS-Tg could serve as the first animal model for understanding the ~~ESRD~~AGE-RAGE-mediated process of diabetogenic kidney pathology. Thus, the AGE-RAGE system may be a promising target for preventive and therapeutic methods of diabetic complications.

Please replace the paragraph beginning on page 5, line 20 with the following rewritten paragraph:

In addition, since ~~the models of the present invention~~ mesangial matrix expansion is accelerated by mesangial cell disorder (megsin overexpression) and glomerular disorders are detected at an early stage in the models of the present invention, the models have features that allow a more detailed comparison and analysis of the differences in the megsin pathophysiology and mesangial cell

pathophysiology in diabetic nephropathy over a wide range of progressional stages of the pathology. Thus, the model animals of the present invention are useful as an evaluation model for developing novel diagnostic methods and drugs that suppress the progression of kidney disorder before late-stage pathologies occur.

Please replace the paragraph beginning on page 5, line 31 with the following rewritten paragraph:

[1] A disease model animal overexpressing megsin gene, a gene encoding the receptor for advanced glycation end-products, and an inducible nitric oxide synthase gene, wherein the model animal comprises a nonhuman mammal.

Please replace the paragraph beginning on page 6, line 1 with the following rewritten paragraph:

[3] The disease model animal of [1] or [2], which exhibits at least one phenotype selected from the following phenotypes (a) to ~~(f)~~(g):

(a) increase in kidney-to-body weight ratio;

(b) increase in urine albumin level;

(c) increase in blood triglyceride level;

(d) underweight (hypogenesis);

(e) hyperglycemia;~~ and~~

(f) hypoinsulinemia~~.~~; and

(g) increase in urine 8-OHdG level.

Please replace the paragraph beginning on page 8, line 3 with the following rewritten paragraph:

(2) determining the glucose ~~or~~/and/or insulin level in the disease model animal after administration of the test compound.

Please replace the paragraph beginning on page 12, line 4 with the following rewritten paragraph:

In the creation of transgenic animals, it is beneficial to ligate each of the genes described above with a promoter that can express the gene in animal cells to be introduced with. The chicken β actin promoter which can express foreign genes in a broad range of vertebrates, including mice and rats, can be used. Alternatively, when a gene is to be expressed in a specific tissue, a tissue-specific promoter may be used. For example, the promoter of mouse flk-1 is a vascular endothelial cell-specific promoter. If required, an enhancer may be used in combination to enhance the expression of a foreign gene. A conventional vector (for example, pCAGGS or such) that comprises an enhancer and a promoter, and downstream thereof, a multi-cloning site for insertion of foreign genes may be used. In these vectors, a rabbit β globin terminator is placed downstream of the multi-cloning site, and this improves the expression efficiency of the inserted foreign genes.

Please replace the paragraph beginning on page 12, line 15 with the following rewritten paragraph:

Transgenic animals are created using each of the genes ligated with a promoter described above. The disease model animals of the present invention comprise a triple transgenic animal introduced with the three genes described above (megsin, RAGE, and iNOS genes). The triple transgenic animal may be created by introducing these three genes into one germinal cell and ~~strongly~~ overexpressing the three genes. Alternatively, each gene may be separately introduced into a different germinal cell and then the resulting respective transgenic animals are crossed sequentially to create a triple Tg animal having the three foreign genes. The method for creating the transgenic animal of the present invention is not limited to specific methods, and art-known methods for creating transgenic animals (see, for example, "Hassei Kohgaku Jikken Manyuaru (Manual for Experiments in Development Engineering)", ed. M. Katsuki (Japan, Kodansha), 1989; "Shin Seikagaku Jikken Kohza, Doubutsu Jikkenhou (New series, Lecture for Biochemical Experiments: Methods of Animal Experiments)" ed. The Japanese Biochemical Society, (Japan, Tokyo Kagakudojin) 1991) may be used. A general protocol for transgenic animal preparation is briefly described below.

Please replace the paragraph beginning on page 17, line 24 with the following rewritten paragraph:

The second evaluation method uses as an indicator a clinical marker associated with glomerular disorders, such as diabetic nephropathy, comprising the steps of: administering a test compound to the above-described megsin/RAGE/iNOS-expressing disease model animal and measuring at least any one of the kidney-to-body weight ratio, level of proteinuria ~~level~~, and blood triglyceride concentration in the disease model animal after administration of the test compound. Specifically, in this method, the therapeutic effect on kidney function disorders is evaluated based on one or more indicators of increased kidney-to-body weight ratio, proteinuria, and blood triglyceride .

Please replace the paragraph beginning on page 17, line 32 with the following rewritten paragraph:

In the second evaluation method described above, first, a test compound is administered to a megsin/RAGE/iNOS-expressing model animal of the present invention. As described above, there is no particular limitation to this route. The kidney functions of the disease model animal treated by the administration and a control model animal that has not been administered with the test compound are compared based on the indices described above. Furthermore, the model animal may be compared with healthy animal subjects. The method for measuring the ~~increase in~~ kidney-to-body weight ratio, level of proteinuria, and blood triglyceride concentration is the same as described above. In comparison with an untreated group, a test compound which can relieve kidney disorder or restore kidney function to the level of a normal animal when administered can be a therapeutic agent for accompanying kidney function disorders of glomerular failure, such as diabetic nephropathy.

Please replace the paragraph beginning on page 24, line 1 with the following rewritten paragraph:

[Example 4] Detection of deposition ~~Deposition of~~ immune complexes by immunofluorescence microscopy

Please replace the paragraph beginning on page 24, line 28 with the following rewritten paragraph:

In the transgenic animals of the present invention, the remodeling of ~~a~~ mesangial matrix that has expanded due to megsin overexpression was impaired rapidly, and this aggravated the

glomerular disorder and shortened the time until the onset of sclerosis. It is also presumed that the glomeruli of the megsin/RAGE/iNOS-Tg animals are composed of mesangial cells exposed to burdens such as ~~high~~ overexpression of megsin ~~expression~~ and hyperglycemia, and endothelial cells under oxidative stress. When only the mesangial cells were exposed to the burdens of megsin overexpression and hyperglycemia, the glomerular disorder is very mild (megsin/iNOS-Tg). However, if the endothelial cells are also exposed to burdens such as activation of the AGE-RAGE pathway, specifically, strong oxidative stress and various cytokines the clinical condition is very mild in the megsin/RAGE/iNOS-Tg mice (Tg mice in which endothelial cells are not under the burden of AGE-RAGE pathway activation) whose glucose level is normal. The megsin/RAGE/iNOS-Tg mouse model suggest that cross talk between the mesangial cells and endothelial cells influences the pathological development.

Please replace the paragraph beginning on page 25, line 24 with the following rewritten paragraph:

[Example 7] Acceleration of mesangial matrix expansion as a result of ~~high~~ overexpression of megsin ~~expression~~

Please replace the paragraph beginning on page 26, line 5 with the following rewritten paragraph:

In comparison with the wild type and megsin-Tg mice, glomerular hypertrophy was found in both the RAGE/iNOS-Tg mice and the megsin/RAGE/iNOS-Tg mice at 16 weeks old (Fig. 9 (a)). ~~High~~ Overexpression of megsin ~~expression~~ did not accelerate glomerular hypertrophy in the RAGE/iNOS-Tg mice. At 16 weeks old, the wild–type mice, megsin-Tg mice, and RAGE/iNOS-Tg mice were revealed to be indifferent in the size of their mesangial matrices. However, expansion of the mesangial matrix was notably accelerated by ~~high~~ overexpression of megsin ~~expression~~ in the megsin/RAGE/iNOS-Tg mice (Fig. 9(b)). These findings correlate with the development of severe mesangial matrix expansion and nodular lesion in the megsin/RAGE/iNOS-Tg mice in comparison with the RAGE/iNOS-Tg mice.

Please replace the paragraph beginning on page 26, line 21 with the following rewritten paragraph:

[Example 8] Early-stage proliferation of glomerular cells by ~~high~~ overexpression of megsin ~~expression~~

Please replace the paragraph beginning on page 27, line 12 with the following rewritten paragraph:

Recent studies have suggested that oxidative stress plays a pathogenic role in diabetic nephropathy. Thus, the present inventors compared the state of oxidative stress between the RAGE/iNOS-Tg and megsin/RAGE/iNOS-Tg mice. These mouse subjects were tested for their urine 8-OHdG, which is an oxidative stress marker. 8-OHdG was detected using an ELISA kit (8-OHdG Check (High sensitive); Japan Aging Control laboratories; Shizuoka, Japan). The urine used in the test was treated by ultrafiltration using Vivaspin (>10, 000 molecular weight cutoffs; Vivascience AG; Hannover, Germany), and the level of 8-OHdG in urine was then determined. The values determined were normalized using urine ~~creatine~~creatinine. The result is shown in Fig. 12.

Please replace the paragraph beginning on page 28, line 20 with the following rewritten paragraph:

In addition, since the incidence of the late-stage pathologies is high, the mice are useful for elucidation of the onset/progression mechanism of diabetic nephropathy and drug discovery. Furthermore, the model animals are useful for developing pharmaceuticals to treat accompanying kidney function disorders of the above-described glomerular failure. The present application provides not only the disease model animals but also a system for evaluating therapeutic agents for kidney function disorder using the model animals. Thus, the present invention is highly useful in clinical studies and development of pharmaceuticals for ~~diseases that accompany kidney function disorders~~ the accompanying kidney function disorders of the above-described glomerular failure. Furthermore, the disease model animals of the present invention exhibit hyperglycemia, and thus are also expected to be highly useful in clinical studies and development of therapeutic and preventive agents for diabetes mellitus.